CLAIMS

What is claimed is:

1. A method for cloning a non-human mammal through a nuclear transfer process comprising:

(i) obtaining desired differentiated mammalian cells to be used as a source of donor nuclei;

(ii) obtaining at least one oocyte from a mammal of the same species as the cells which are the source of donor nuclei;

(iii) enucleating said at least one oocyte;

(iv) transferring the desired differentiated cell or cell nucleus into the enucleated oocyte;

(v) simultaneously fusing and activating the cell couplet to form a first transgenic embryo;

(vi) activating a cell-couplet that does not fuse to create a first transgenic embryo but that is activated after an initial electrical shock by providing at least one additional activation protocol including an additional electrical shock to form a second transgenic embryo;

(vii) culturing said activated first and/or second transgenic embryo(es) until greater than the 2-cell developmental stage; and

(viii) transferring said first and/or second transgenic embryo into a host mammal such that the embryo develops into a fetus.

2. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from mesoderm.

3. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from endoderm.

4. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from ectoderm.

5. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from fetal somatic tissue.

6. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from fetal somatic cells.

7. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from a fibroblast.

8. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an ungulate.

9. The method of either claims 1 or 8, wherein said donor cell or donor cell nucleus is from an ungulate selected from the group consisting of bovine, ovine, porcine, equine, caprine and buffalo.

10. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an adult non-human mammalian somatic cell.

11. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is selected from the group consisting of epithelial cells, neural cells, epidermal cells, keratinocytes, hematopoietic cells, melanocytes, chondrocytes, B-lymphocytes, T-lymphocytes, erythrocytes, macrophages, monocytes, fibroblasts, and muscle cells.

12. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an organ selected from the group consisting of skin, lung, pancreas, liver, stomach, intestine, heart, reproductive organ, bladder, kidney and urethra.

13. The method of claim 1, wherein said at least one oocyte is matured *in vivo* prior to enucleation.

14. The method of claim 1, wherein said at least one oocyte is matured *in vitro* prior to enucleation.

15. The method of claim 1, wherein said non-human mammal is a rodent.

16. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is a non-quiescent somatic cell or a nucleus isolated from said non-quiescent somatic cell.

17. The method of either claims 1 or 8, wherein the fetus develops into an offspring.

18. The method of claim 1, wherein said at least one oocyte is enucleated about 10 to 60 hours after initiation of *in vitro* maturation.

19. The method of claim 1, wherein a desired gene is inserted, removed or modified in said differentiated mammalian cell or cell nucleus prior to insertion of said differentiated mammalian cell or cell nucleus into said enucleated oocyte.

20. The resultant offspring of the methods of claims 1 or 19.

21. The resultant offspring of claim 19 further comprising wherein the offspring created as a result of said nuclear transfer procedure is chimeric.

22. The method of claim 1, wherein cytocholasin-B is used in the cloning protocol.

23. The method of claim 1, wherein cytocholasin-B is not used in the cloning protocol.

24. A method for producing cultured inner cell mass cells, comprising:

(i) obtaining desired differentiated mammalian cells to be used as a source of donor nuclei;

(ii) obtaining at least one oocyte from a mammal of the same species as the cells which are the source of donor nuclei;

(iii) enucleating said at least one oocyte;

(iv) transferring the desired differentiated cell or cell nucleus into the enucleated oocyte;

(v) simultaneously fusing and activating the cell couplet to form a first transgenic embryo;

(vi) activating a cell-couplet that does not fuse to create a first transgenic embryo but that is activated after an initial electrical shock by providing at least one additional activation protocol including an additional electrical shock to form a second transgenic embryo; and

(vi) culturing cells obtained from said cultured activated embryo to obtain cultured inner cell mass cells.

25. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from mesoderm.

26. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from endoderm.

27. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from ectoderm.

28. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from fetal somatic tissue.

29. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from fetal somatic cells.

30. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from a fibroblast.

31. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an ungulate.

32. The method of either claims 24 or 31, wherein said donor cell or donor cell nucleus is from an ungulate selected from the group consisting of bovine, ovine, porcine, equine, caprine and buffalo.

33. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an adult mammalian somatic cell.

34. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is selected from the group consisting of epithelial cells, neural cells, epidermal cells, keratinocytes, hematopoietic cells, melanocytes, chondrocytes, B-lymphocytes, T-lymphocytes, erythrocytes, macrophages, monocytes, fibroblasts, and muscle cells.

35. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an organ selected from the group consisting of skin, lung, pancreas, liver, stomach, intestine, heart, reproductive organ, bladder, kidney and urethra.

36. The method of claim 24, wherein said at least one oocyte is matured *in vivo* prior to enucleation.

37. The method of claim 24, wherein said at least one oocyte is matured *in vitro* prior to enucleation.

38. The method of claim 24, wherein said mammalian cell is derived from a rodent.

39. The method of claim 24, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is a non-quiescent somatic cell or a nucleus isolated from said non-quiescent somatic cell.

40. The method of either claims 24 or 31, wherein any of said cultured inner cell mass cells fetus develops into a non-human offspring.

41. The method of claim 24, wherein said at least one oocyte is enucleated about 10 to 60 hours after initiation of *in vitro* maturation.

42. The method of claim 24, wherein a desired gene is inserted, removed or modified in said differentiated mammalian cell or cell nucleus prior to insertion of said differentiated mammalian cell or cell nucleus into said enucleated oocyte.

43. The resultant offspring of the methods of claims 24 or 42.

44. The resultant offspring of claim 42 further comprising wherein any non-human offspring created as a result of said nuclear transfer procedure is chimeric.

45. The method of claim 24, wherein cytocholasin-B is used in the protocol.

46. The method of claim 24, wherein cytocholasin-B is not used in the protocol.

47. The method of claim 24, wherein cytocholasin-B is used in the protocol.

48. The method of claim 24, wherein said cultured inner cell mass cells are used to develop a functional organ for transplantation.

49. The method of claim 24, wherein said cultured inner cell mass cells are used in organogenesis.

50. A method for cloning a non-human mammal through a nuclear transfer process comprising:

(i) obtaining desired differentiated mammalian cells to be used as a source of donor nuclei;

(ii) obtaining at least one oocyte from a mammal of the same species as the cells which are the source of donor nuclei;

(iii) enucleating said oocytes;

(iv) transferring the desired differentiated cell or cell nucleus into the enucleated oocyte;

employing at least two electrical shocks to a cell-couplet to initiate fusion and activation of said cell-couplet into an activated and fused embryo.

(vii) culturing said activated and fused embryo until greater than the 2-cell developmental stage;

(viii) transferring said first and/or second transgenic embryo into a host mammal such that the embryo develops into a fetus;

wherein the second of said at least two electrical shocks is administered at least 15 minutes after an initial electrical shock; and

wherein a desired gene is inserted, removed or modified in said differentiated mammalian cell or cell nucleus prior to insertion of said differentiated mammalian cell or cell nucleus into said enucleated oocyte.